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Survival of *Clavibacter michiganensis* ssp. *michiganensis* in infected tomato stems under natural field conditions in California, Ohio and Morocco

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The survival and half-life of *Clavibacter michiganensis* ssp. *michiganensis* (*C. michiganensis*), the causal agent of bacterial canker of tomato, were determined in infected plant debris under natural field conditions in California, Ohio and Morocco using a semiselective agar medium. The organism survived significantly longer in tomato stems left on the soil surface than in stems buried in the soil at all locations studied. The pathogen was recovered in high amounts from tomato stems left on the soil surface for 314 days in Ohio and California, USA, and for 194 and 132 days in Melk Zhar and Aït Melloul, Morocco, respectively; it was recovered from stems buried in the soil for up to 314 days in Ohio, up to 240 days in California, and up to 60 days in Aït Melloul and Melk Zhar. The half-life of the pathogen in stems left on the soil surface ranged from 23·2 to 24·8 days in the USA, and from 7·8 to 12·3 days in Morocco, whereas the half-life in buried stems ranged from 14·0 to 16·7 days in the USA and from 3·7 to 9·5 days in Morocco. Based on the half-life data, the predicted survival times of *C. michiganensis* in stems on the soil surface in Ohio, California, Melk Zhar and Aït Melloul would be up to 822, 770, 424 and 261 days, respectively, while the predicted survival times in stems buried in the soil would be 541, 497, 305 and 128 days, respectively. These results show that the survival and half-life of *C. michiganensis* in plant debris are relatively long and are influenced by both tissue exposure and geographic location.

Keywords: *Clavibacter michiganensis* ssp. *michiganensis*, survival, tomato debris

Introduction

Bacterial canker of tomato, caused by *Clavibacter michiganensis* ssp. *michiganensis* (Davis *et al*., 1984) (*C. michiganensis*), is one of the most destructive diseases of tomato (Bryan, 1930; Ark, 1944; Ark & Thompson, 1960). *Clavibacter michiganensis* survives in seed (Bryan, 1930; Grogan & Kendrick, 1953; Patino-Mendez, 1964) and soil (Bryan, 1930; Grogan & Kendrick, 1953; Strider, 1967; Farley, 1971; Moffet & Wood, 1984) and has several alternative hosts (Baines, 1947; Ark & Thompson, 1960; Strider, 1967; Thyr, 1971; Thyr *et al*., 1975). Data on the longevity of *C. michiganensis* in soil differ, because of differences in location and detection methods (Bryan, 1930; Strider, 1967; Basu, 1970; Echandi, 1971; Moffet & Wood, 1984). Basu (1970) reported a survival period of 9 months in tomato leaflets buried in soil at –20°C, but only 3 weeks at 5–35°C. Bryan (1930) detected the organism in infested soil tubes after outdoor incubation for 11 months in Washington, DC, and New York. In addition, Strider (1967) detected *C. michiganensis* in infested soil after 18 months' exposure to outdoor temperatures in North Carolina. Ciccarone & Carilli (1948) reported the survival of *C. michiganensis* in soil in the field for 4 years in Italy. Many authors (Grogan & Kendrick, 1953; Strider, 1967; Fahy & Hayward, 1983) reported survival in host tissue in soil exposed to winter freezing. Other reports (Ciccarone & Carilli, 1948; Farley, 1971) suggest that *C. michiganensis* persisted in soil indefinitely. In the above studies, the prevalence or existence of *C. michiganensis* in soil was not based on isolation of the organism, but solely on observations of disease occurring on tomato plants in subsequent plantings (Bryan, 1930; Ciccarone & Carilli, 1948; Grogan & Kendrick, 1953; Strider, 1967; Basu, 1970; Echandi, 1971; Farley, 1971). Trevors & Finnen (1990) recovered cells of *C. michiganensis* after 1 month in natural field soil, but the soil was placed in Petri dishes under controlled conditions, not left in the field. Moffet & Wood (1984) recovered *C. michiganensis* from host tissue in the field in Australia after 3 and 7 months, using a rifampin-marked mutant strain. Gleason *et al.* (1991) reported survival of rifampin-marked strains for 24 months in debris on the soil surface and 7 months in

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debris buried in soil in Iowa, USA. Similarly, Chang *et al.* (1992) detected approximately 10^6 and 10^4 cfu (colony-forming units) of rifampin-marked strains per g of buried debris in Illinois after 196 and 210 days in 1988–89 and 1989–90, respectively.

The field studies reported here were conducted from November 1985 to September 1986 in the USA and from March to September 1987 in Morocco. The purpose of this study was to determine quantitatively the survival of *C. michiganensis* in tomato stems in soil under widely different field conditions based upon a laboratory assay using unaltered wild-type strains of *C. michiganensis* and a semiselective agar medium.

Materials and methods

Source of inoculum and infected tomato stems

For the studies in California and Ohio, USA, stems from inoculated plants were used. Early log-phase cells were obtained by growing *C. michiganensis* strain H-122 (Fatmi & Schaad, 1988) in liquid nutrient broth yeast extract (NBY) medium (Gross & Vidaver, 1979) on a New Brunswick G-25 rotary incubator shaker for 24 h at 26°C. Suspensions were adjusted to 0·1 optical density at 640 nm using a Spectronic 20 colorimeter and diluted 10-fold in water. Thirty to 50 μL of the suspension, containing approximately 10^8 cfu of *C. michiganensis* per mL, were injected into stems of 30 glasshouse-grown tomato (cv. Glamour) plants at the second or third true-leaf sage, as described by Moffet *et al.* (1983). Strain H-122 was chosen because of its high virulence and high recovery on SCM medium (Fatmi & Schaad, 1988). After 2 months in the glasshouse (maintained at 27–30°C), stems 10 cm long and 1 cm in diameter from 24 inoculated tomato plants exhibiting bacterial canker symptoms were excised and stored at 4°C. After sampling as described below, 12 stems were sent overnight to Davis, California, and 12 to Napolean, Ohio, USA. For the study in Morocco, tomato stems about 15 cm long and 1·5 cm in diameter were excised from 24 naturally diseased mature tomato plants growing on several different farms in the Souss-Massa region (Agadir) and stored at 4°C. After sampling the next day, as described below, eight stems were left on the soil surface and 16 were buried in the field.

Determination of the original inoculum of the selected tomato stems

The number of cfu of *C. michiganensis* per g dry weight of tissue for each of the tomato stems selected was determined by cutting two 1-mm cross-sections from the ends of each stem (Schaad & White, 1974). One section of each pair from each end was used to determine dry weight (after drying at 70°C for 7 days). The other section from each end was comminuted in 9 mL of sterile phosphate buffer (0·05 M PO_4, pH 7·4) using a sterile pestle and mortar, diluted serially in 10-fold dilutions to 10^{-7} in the same buffer, and 10^{-6} and 10^{-7} dilutions assayed in triplicate on SCM agar plates (Fatmi & Schaad, 1988). The plates were incubated at 26°C for 8–9 days. Plates with 50–200 colonies each were used for the counting of colony numbers. Suspected colonies of *C. michiganensis* with typical morphologies were purified by streaking onto plates of NBY agar medium and tested for Gram staining (Fahy & Hayward, 1983). Pathogenicity was determined by inoculating stems of susceptible tomato plants at the second or third true-leaf stage (Moffet *et al.*, 1983).

Location

Stems were wrapped individually in a piece of nylon netting and divided randomly into 12 samples. Four samples (replicates) were left on the soil surface and eight were buried at each location. Experiments were initiated on 6 November 1985 in Riff sandy loam soil at the Campbell Institute for Research and Technology, Davis, California; on 17 November 1985 in Millgrove loam soil at the Campbell Institute for Research and Technology, Napoleon, Ohio; on 1 March 1987 in sandy soil at Melk Zhar INRA (Institut National de Recherche Agronomique) Station in Massa, Agadir, Morocco; and on 1 March 1987 in clay loam soil at Institut Agronomique et Vétérinaire Hassan II, Complexe Horticole d'Agadir, Morocco. Samples of soil from each of the four locations were collected prior to burial of the stems. At each location, four stem replicates were placed on the soil surface and eight stem replicates were buried 10 cm below the soil surface at two different sites 150 m apart. All stems were transported to and from the field on ice, except for the overnight shipment for the California and Ohio samples.

Assay of stem samples

The stems in California and Ohio were recovered and express-mailed to Moscow, Idaho, USA. The samples in California were assayed every 2 months, while the stems in Ohio were assayed after the ground had thawed (17 March 1986) and every 2 months thereafter. Sections of tissue were removed and assayed in the same way as the original samples above (except that dilutions from 10^{-2} to 10^{-7} were included) and returned to the test sites in California and Ohio within 24 h. The stems buried in Morocco were brought by car to the laboratory at Institut Agronomique et Vétérinaire Hassan II in Aït Melloul, Agadir, assayed as above, and returned the same day. Stems at locations in Morocco were assayed approximately every month. To determine if negative results might be due to saprophytic bacteria inhibiting growth of *C. michiganensis*, one plate of each stem sample from which no *C. michiganensis* was recovered was oversprayed after 8 days with a 100-fold dilution of a 48-h liquid culture of *C. michiganensis* H112 (Randhawa & Schaad, 1984). The experiment was terminated after 314 and 224 days in the USA and Morocco, respectively, and the half-life (defined as the time for a population to lose one-half of its viability) of *C. michiganensis* determined as described by Yarwood & Sylvester (1959).

Identification of isolated colonies

Several colonies suspected of being *C. michiganensis,* based on colony morphology on SCM agar, were cloned by streaking onto NBY agar and tested for pathogenicity, as described above.

Results

Assays of soil samples collected from the four sites prior to setting out the tissue samples were negative for *C. michiganensis*.

Survival on soil surface

From 6 November 1985 to 20 September 1986 and from 17 November 1985 to 30 September 1986, the mean number of cfu of *C. michiganensis* per g dry weight of tissue decreased from $9{\cdot}1 \times 10^9$ to $7{\cdot}8 \times 10^5$, and from $9{\cdot}6 \times 10^9$ to $1{\cdot}4 \times 10^6$ at Davis, California, and Napoleon, Ohio, respectively (Table 1). At Melk Zhar, from 1 March to 10 September 1987 (194 days), the mean number of cfu of *C. michiganensis* per g dry weight of tissue decreased from $2{\cdot}4 \times 10^{10}$–$4{\cdot}5 \times 10^5$ (Table 1). At Aït Melloul, from 1 March to 10 July 1987 (132 days), the mean number of cfu of *C. michiganensis* per g dry weight of tissue decreased from $1{\cdot}2 \times 10^{10}$–$9{\cdot}5 \times 10^4$ (Table 1). The half-lives of the pathogen in stem tissue placed on the soil surface at Davis, Napoleon, Melk Zhar and Aït Melloul were 23·2, 24·8, 12·3 and 7·8 days, respectively (Table 2). Predicted survival times ranged from a high of 822 days in Ohio to a low of 261 days at Aït Melloul, Morocco (Table 2). No antagonistic bacteria were detected on any of the control over-spray plates.

Survival in buried stems

At Davis, California, the mean number of cfu of *C. michiganensis* per g dry weight of tissue declined from $9{\cdot}1 \times 10^9$ to $6{\cdot}1 \times 10^4$ after 240 days at site I and from $1{\cdot}8 \times 10^{10}$–$3{\cdot}5 \times 10^6$ after 180 days at site II (Table 1). At Napoleon, Ohio, the mean cfu of *C. michiganensis* dropped from $5{\cdot}6 \times 10^9$ to $1{\cdot}2 \times 10^4$ after 314 days at site I and from $8{\cdot}0 \times 10^9$ to $6{\cdot}8 \times 10^4$ after 240 days at site II (Table 1). At Melk Zhar, the mean number of cfu of *C. michiganensis* per g dry weight of tissue decreased from $1{\cdot}6 \times 10^{10}$ to $1{\cdot}2 \times 10^6$ after 60 days at site I, and from $4{\cdot}7 \times 10^9$ to $6{\cdot}0 \times 10^7$ after 60 days at site II (Table 1). At Aït Melloul, after 60 days, the mean cfu of *C. michiganensis* decreased from $8{\cdot}8 \times 10^9$ to $1{\cdot}1 \times 10^5$ at site I, and from $1{\cdot}3 \times 10^{10}$ to $2{\cdot}1 \times 10^5$ at site II (Table 2). Half-lives of the pathogen in stems buried at sites I and site II at Davis, Napoleon, Melk Zhar and Aït Melloul were 14·0 and 14·5, 16·7 and 14·2, 4·4 and 9·5, and 3·7 and 3·8 days, respectively (Table 2). Predicted survival times ranged from a high of 541 days at one site in Ohio to a low of 122 days at one site in Aït Melloul, Morocco. No antagonistic bacteria were observed in any of the Ohio or California samples. However, antagonistic bacteria were observed in 93-day Melk Zhar samples. No growth of *C. michiganensis* was observed on the control over-spray plate for the 93-day samples at Melk Zhar (Table 1).

All suspect colonies from stems either on the soil surface or buried were pathogenic on tomato.

Discussion

Using SCM agar medium (Fatmi & Schaad, 1988), *C. michiganensis* was found to survive for long periods in infected tomato stems. Survival varied with location and according to whether the stems were left on the soil surface or buried. This variation might be due to several factors, including climate (Basu, 1970), soil type (Moffet & Wood, 1984) and soil microflora.

Survival of bacteria for 26 months in stems on the soil surface in Ohio is similar to the longevity reported by Gleason *et al.* (1991) in Iowa, but the predicted survival of 18 months for buried stems in this study differed from the 7 months reported in that study. These results clearly show the potential for long-term survival of *C. michiganensis* in infected tomato stem or leaf materials. The relatively long survival period in soil agrees with the conclusion that the use of 'clean' seeds alone will not control the disease under field conditions (Gleason *et al.*, 1991; Chang *et al.*, 1992). Also, these results confirm that inoculum originating from infected stem debris may pose a threat under optimum conditions for pathogen spread and development (Gleason *et al.*, 1991). When survival in soil was based on presence or absence of disease in subsequent tomato plantings, the results varied: some studies showed indefinite survival (Ciccarone & Carilli, 1948; Farley, 1971), whereas others (Bryan, 1930; Grogan & Kendrick, 1953; Strider, 1967; Basu, 1970; Echandi, 1971) showed little or no survival. Results of this study agree with those studies using agar plating of mutated marked strains in that *C. michiganensis* survives in debris for long periods. The use of SCM agar and wild-type strains allows the presence of *C. michiganensis* in infected tomato stems to be followed over time, both qualitatively and quantitatively. The diversity in numbers and types of saprophytic bacteria in soils and the possible inability of some strains of *C. michiganensis* to grow efficiently on SCM are limitations on the use of the medium when the population of *C. michiganensis* becomes very low. However, the use of SCM and wild-type strains appeared to be more sensitive than the use of strains with rifampin-resistant mutations (Moffet & Wood, 1984; Gleason *et al.*, 1991; Chang *et al.*, 1992). Rifampin resistance is conferred by missense mutations within DNA-dependent RNA polymerase encoded by the *rPO* β gene (Telenti *et al.*, 1993). The occurrence of natural resistance to rifampin in bacteria (Telenti *et al.*, 1993; Whelen *et al.*, 1995) casts some doubt on the use of *rif* as a unique marker to identify *C. michiganensis*, as many false positives could occur.

Results showing a decrease in the number of cfu of *C. michiganensis* at Davis, California, in contrast to a stable population at Napoleon, Ohio, for the first 120 days, were most probably due to the weather, since the stems in

Table 1 Survival of *Clavibacter michiganensis* ssp. *michiganensis* in tomato stem tissue under natural conditions in California and Ohio, USA, and Melk Zhar and Aït Melloul, Morocco

		Mean colony-forming units (cfu) of *C. michiganenis* per g dry weight of tissue[a]		
			Buried	
Location	Days	Soil surface	Site I	Site II
California	0	$9{\cdot}1 \times 10^9 \pm 3{\cdot}5$[b]	$9{\cdot}1 \times 10^9 \pm 2{\cdot}7$	$1{\cdot}8 \times 10^{10} \pm 1{\cdot}0$
	60	$6{\cdot}1 \times 10^9 \pm 7{\cdot}5$	$6{\cdot}7 \times 10^7 \pm 3{\cdot}8$	$6{\cdot}6 \times 10^8 \pm 6{\cdot}2$
	120	$5{\cdot}5 \times 10^8 \pm 7{\cdot}1$	$5{\cdot}3 \times 10^5 \pm 4{\cdot}7$	$9{\cdot}5 \times 10^5 \pm 12{\cdot}4$
	180	$7{\cdot}8 \times 10^7 \pm 3{\cdot}3$	$7{\cdot}1 \times 10^3 \pm 14{\cdot}3$	$3{\cdot}5 \times 10^6 \pm 6{\cdot}8$
	240	$7{\cdot}9 \times 10^7 \pm 10{\cdot}0$	$6{\cdot}1 \times 10^4 \pm 12{\cdot}2$	0·0
	314	$7{\cdot}8 \times 10^5 \pm 12{\cdot}0$	0·0	0·0
Ohio	0	$9{\cdot}6 \times 10^9 \pm 10{\cdot}9$[b]	$5{\cdot}6 \times 10^9 \pm 2{\cdot}2$	$8{\cdot}0 \times 10^9 \pm 2{\cdot}2$
	60	–[c]	–[c]	–[c]
	120	$4{\cdot}1 \times 10^9 \pm 2{\cdot}9$	$4{\cdot}1 \times 10^8 \pm 4{\cdot}5$	$1{\cdot}6 \times 10^9 \pm 1{\cdot}7$
	180	$6{\cdot}6 \times 10^8 \pm 4{\cdot}3$	$1{\cdot}3 \times 10^7 \pm 1{\cdot}7$	$3{\cdot}5 \times 10^7 \pm 2{\cdot}6$
	240	$8{\cdot}2 \times 10^8 \pm 16{\cdot}4$	$2{\cdot}3 \times 10^5 \pm 3{\cdot}2$	$6{\cdot}8 \times 10^4 \pm 13{\cdot}8$
	314	$1{\cdot}4 \times 10^6 \pm 2{\cdot}6$	$1{\cdot}2 \times 10^4 \pm 2{\cdot}3$	0·0
Melk Zhar	0	$2{\cdot}4 \times 10^{10} \pm 1{\cdot}4$[b]	$1{\cdot}6 \times 10^{10} \pm 1{\cdot}8$	$4{\cdot}7 \times 10^9 \pm 3{\cdot}7$
	30	$1{\cdot}9 \times 10^9 \pm 1{\cdot}3$	0·0	$8{\cdot}6 \times 10^4 \pm 17{\cdot}3$
	60	$5{\cdot}9 \times 10^7 \pm 6{\cdot}7$	$1{\cdot}2 \times 10^6 \pm 2{\cdot}4$	$6{\cdot}0 \times 10^7 \pm 10{\cdot}0$
	93	$1{\cdot}2 \times 10^7 \pm 2{\cdot}3$	0·0[d]	0·0[d]
	132	$1{\cdot}4 \times 10^5 \pm 2{\cdot}6$	0·0	0·0
	194	$4{\cdot}5 \times 10^5 \pm 7{\cdot}8$	0·0	0·0
	224	0·0	0·0	0·0
Aït Melloul	0	$1{\cdot}2 \times 10^{10} \pm 1{\cdot}0$[b]	$8{\cdot}8 \times 10^9 \pm 12{\cdot}1$	$1{\cdot}3 \times 10^{10} \pm 1{\cdot}9$
	30	$1{\cdot}1 \times 10^9 \pm 1{\cdot}5$	0·0	$3{\cdot}3 \times 10^5 \pm 6{\cdot}7$
	60	$3{\cdot}7 \times 10^7 \pm 5{\cdot}8$	$1{\cdot}1 \times 10^5 \pm 2{\cdot}3$	$2{\cdot}1 \times 10^5 \pm 4{\cdot}4$
	93	$1{\cdot}6 \times 10^7 \pm 3{\cdot}4$	0·0	0·0
	132	$9{\cdot}5 \times 10^4 \pm 10{\cdot}0$	0·0	0·0
	194	0·0	0·0	0·0
	224	0·0	0·0	0·0

[a]Data obtained from the mean of two 1-mm cross-sections of tissue, one from each end of the stem piece. Tissue was ground in buffer and assayed by dilution plating on triplicate plates of SCM agar (Fatmi & Schaad, 1988).
[b]The standard deviation is raised to the same order of magnitude as the mean.
[c]Samples were not assayed because of frozen soil.
[d]Control plates over-sprayed with *C. michiganensis* showed the presence of antagonistic colonies.

Table 2 Half-lives and predicted survival of *Clavibacter michiganensis* ssp. *michiganensis* in infected tomato stems at different locations in the USA and Morocco based on extrapolated data

	Half-life (days)[a]			Predicted survival (days)[b]		
		Buried			Buried	
Locations	Soil surface	Site I	Site II	Soil surface	Site I	Site II
California	23·2	14·0	14·5	769·5	462·0	496·3
Ohio	24·8	16·7	14·2	821·8	541·0	469·0
Melk Zhar	12·3	4·4	9·5	424·2	149·4	305·4
Aït Melloul	7·8	3·7	3·8	261·3	122·3	127·8

[a]The half-life of *C. michiganensis* for the different locations was determined as described by Yarwood & Sylvester (1959).
[b]The theoretical predicted survival time of *C. michiganensis* for the different locations was obtained through extrapolation of the data (half-life and numbers of colony-forming units of *C. michiganensis* detected).

Ohio were frozen for most of this period. Although overnight shipping of samples to and from California and Ohio may have resulted in a more rapid decline in populations of *C. michiganensis*, any effects were apparently very minimal because surface survival in Ohio and California was longer than in Morocco.

In most of the stems, especially those in California and Ohio, there was a general and progressive decrease in the population of *C. michiganensis* over time. However, in some of the stems, particularly those used under Moroccan conditions, the number of detectable cfu of *C. michiganensis* dropped, then increased over time. These variations might be due to several factors. Some sections of the stems assayed might have contained varying levels of *C. michiganensis* due to a nonhomogeneous and discontinuous distribution of *C. michiganensis* along the stems. Whereas stems used under Moroccan conditions were selected from naturally infected tomato plants, stems used in the USA were taken from tomato plants inoculated in the glasshouse, and the glasshouse conditions could have resulted in a more uniform infection. Although the strain of *C. michiganensis* used for the California and Ohio experiments had a high plating efficiency on SCM agar, the plating efficiency of the wild-type strains in Morocco were unknown and the plating efficiency could have been less, making recovery of fewer cells more difficult in the latter assays. There could also have been mixed infections of strains with varying recoveries on SCM. The most likely cause of the failure to detect *C. michiganensis* in some assays was the presence of antagonistic bacteria. This was supported by positive results of the over-spray control plate for the 93-day assay for both sites at Melk Zhar (Table 1). Control (unused) plates of SCM over-sprayed with the same culture of *C. michiganensis* always resulted in normal growth. Therefore, the failure to detect *C. michiganensis* in some sections of stems at one particular time does not automatically mean *C. michiganensis* was not present. Also, numbers of cells could have been below the detection threshold. To be sure the pathogen is not present when assay results are negative, stems should be assayed again 1–2 months later. However, this was not possible for some stems due to lack of material.

These results suggest that residues of tomato plants should be ploughed and fields rotated to a non-tomato crop for 2–3 years, depending on the location. Whether or not similar results would be obtained in other tomato-growing areas must be confirmed by experimental evidence.

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References

Ark PA, 1944. Studies on bacterial canker of tomato. *Phytopathology* **34**, 394–400.

Ark PA, Thompson JP, 1960. Additional host for tomato canker organism: *Corynebacterium michiganense*. *Plant Disease Reporter* **44**, 98–9.

Baines RC, 1947. Perennial nightshade a host for *Corynebacterium michiganense* (Abstract). *Phytopathology* **37**, 359.

Basu PK, 1970. Temperature an important factor determining survival of *Corynebacterium michiganense* in soil. *Phytopathology* **60**, 825–7.

Bryan MK, 1930. Studies on bacterial canker of tomato. *Journal of Agricultural Research* **41**, 825–51.

Chang RS, Ries SM, Pataky JK, 1992. Local sources of *Clavibacter michiganensis* ssp. *michiganensis* in the development of bacterial canker of tomato. *Phytopathology* **82**, 553–60.

Ciccarone A, Carilli A, 1948. Osservazion di campo su *Corynebacterium michiganense* (Smith) Jensen e considerazioni su un possible cao di sua supravvivenza nel terreno. *Bollettino Della Stazione Di Patologia a Vegetale* **6**, 277–80.

Davis MJ, Gillaspie AG Jr, Vidaver AK, Harris RW, 1984. *Clavibacter*: a new genus containing some phytopathogenic Coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. & *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause Ratoon stunting disease of sugarcane and Bermudagrass stunting disease. *International Journal of Systemic Bacteriology* **34**, 107–17.

Echandi E, 1971. Survival of *Corynebacterium michiganense* in soil as free cells in infected tomato tissue (abstract). *Phytopathology* **61**, 890.

Fahy PC, Hayward AC, 1983. Media and methods for isolation and diagnostic tests. In: Fahy, PC, Persley, GJ, eds. *Plant Bacterial Diseases. A Diagnostic Guide*. London, UK: Academic Press, 337–78.

Farley JD, 1971. Recovery of *Corynebacterium michiganense* from overwintered tomato stems by the excised-petiole inoculation method. *Plant Disease Reporter* **55**, 654–6.

Fatmi M, Schaad NW, 1988. A semiselective agar medium for isolation of *Clavibacter michiganensis* subsp. *michiganensis* from tomato seed. *Phytopathology* **78**, 121–6.

Gleason ML, Braun EJ, Carlton WM, Peterson RH, 1991. Survival and dissemination of *Clavibacter michiganensis* subsp. *michiganensis* in tomatoes. *Phytopathology* **81**, 1519–23.

Grogan RG, Kendrick JB, 1953. Seed transmission, mode of overwintering and spread of bacterial canker of tomato caused by *Corynebacterium michiganense* (abstract). *Phytopathology* **43**, 473.

Gross DC, Vidaver AK, 1979. A semiselective medium for isolation of *Corynebacterium nebraskense* from soil and plant parts. *Phytopathology* **69**, 82–7.

Moffet ML, Fahy PD, Cartwright D, 1983. *Corynebacterium*. In: Fahy, PC, Persley, GJ, eds. *Plant Bacterial Diseases. A Diagnostic Guide*. London, UK: Academic Press, 45–65.

Moffet ML, Wood BA, 1984. Survival of *Corynebacterium michiganense* subsp. *michiganense* within host debris in soil. *Australian Plant Pathology* **13**, 1–3.

Patino-Mendez G, 1964. *Studies on the Pathogenicity of* Corynebacterium michiganense *(E. F. Smith) Jensen and its Transmission into Tomato Seed.* PhD Thesis. Davis, CA, USA: University of California.

Randhawa P, Schaad NW, 1984. Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. *Phytopathology* **74**, 268–72.

Schaad NW, White WC, 1974. Survival of *Xanthomonas campestris* in soil. *Phytopathology* **64**, 1518–20.

Strider DL, 1967. Survival studies with the tomato bacterial canker organism. *Phytopathology* **57**, 1067–71.

Telenti A, Imboden P, Marchesi F, Lowrie D, Cole ST, Colston MJ, Matter L, Schopfer K, Bodmer T, 1993. Detection of rifampin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* **341**, 647–50.

Thyr BD, 1971. *Corynebacterium michiganense* isolated from naturally infected *Solanum trifolium*. *Plant Disease Reporter* **55**, 336–7.

Thyr BD, Samuel MJ, Brown PG, 1975. New solanaceous host records for *Corynebacterium michiganense*. *Plant Disease Reporter* **59**, 595–8.

Trevors JT, Finnen RL, 1990. Introduction and recovery of *Clavibacter michiganensis* subsp. *michiganensis* from agricultural soil. *Plant and Soil* **126**, 141–3.

Whelen AC, Felmlee TA, Hunt JM, Williams DL, Roberts GD, Stockman L, Persing DH, 1995. Direct genotypic detection of *Mycobacterium tuberculosis* rifampin resistance in clinical specimens by using single-tube heminested PCR. *Journal of Clinical Microbiology* **33**, 556–61.

Yarwood CE, Sylvester ES, 1959. The half-life concept of longevity of plant pathogens. *Plant Disease Reporter* **43**, 125–8.